Initials: DES/VJT/dcm Docket No.: 1062.2002-004 Date: July 26, 2004

This is to acknowledge receipt of the following documents each filed under Certificate of Mailing Procedure 37 C.F.R. 1.8:

_X_ Trans. of _X_ Seq List w/Pre Amd ___ Sub Seq List w/Pre Amd ___w/copy
_X_ Sequence Listing _X_ Paper _X_ CRF
_X_ Copy of Notice to File Missing Parts
___ Amendment Fee Letter w/copy
___ Petition for Extension of Time
___ Check for $[ ] _____Authorization to Charge all Fees
___ Other_____

Applicants: Ellis L. Reinherz, *et al.*
Application No.: 10/783,994 Filed: February 20, 2004
Title: Identification of the...
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Ellis L. Reinherz, Linda K. Clayton, Emma Fiorini, Pedro A. Reche and Ingo Schmitz

Application No.: 10/783,994 Group: 1632

Filed: February 20, 2004 Examiner: Not yet assigned

Confirmation No.: 4354

For: IDENTIFICATION OF THE IKBNS PROTEIN AND ITS PRODUCTS

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as First Class Mail in an envelope addressed to Mail Stop Sequence, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450

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TRANSMITTAL OF SEQUENCE LISTING AND PRELIMINARY AMENDMENT IN REPLY TO NOTICE TO FILE MISSING PARTS

Mail Stop Sequence
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

This paper is submitted in response to the Notice to File Missing Parts of Application, mailed from the Patent Office on May 26, 2004. A copy of the Notice is attached.

COPY

Transmitted herewith is a copy of the "Sequence Listing" (sheets 1/13 through 13/13), comprising SEQ ID NOS: 1-12 in paper form for the above-referenced patent application as required by 37 C.F.R. §1.821(c) and a copy of the "Sequence Listing" in computer readable form as required by 37 C.F.R. §1.821(e). Please insert the attached "Sequence Listing" into the application.

As required by 37 C.F.R. §1.821(f), Applicant's Attorney hereby states that the content of the "Sequence Listing" in paper form and the computer readable form of the "Sequence Listing" are the same and, as required by 37 C.F.R. §1.821(g), also states that the submission includes no new matter.

COPY

Amendments to the Specification

Please replace the paragraph at page 7, line 19 through page 8, line 7 with the following amended paragraph:

Figure 2 shows the multiple sequence alignment with IκBNS (SEQ ID NO: 4). The full sequence of IκBNS is shown. Numbering refers to IκBNS. The ankyrin domains of IκBNS are boxed and labeled from A to G. Secondary structure (ss) predictions for IκBNS are shown above the alignment with the inner helix of the ankyrin repeat core shown in gray, and the outer in blue. Secondary structure motifs of IκBα were obtained from pdb 1NFI (Jacobs and Harrison, 1998) and are shown below the alignment. Sequences were aligned using the program ClustalX (Thompson *et al.*, 1997), and the secondary structure prediction of IκBNS was determined using PSI-PRED (Jones, 1999). A dendrogram of the figure is displayed with IκBα as the root and was derived using the Neighborjoining method (Saitou and Nei, 1987). Amino acid positions with identities or similarities in 5 or more of the 6 proteins aligned are highlighted in black with yellow letters. Amino acid positions with identities or similarities in four or more of the 6 proteins are highlighted in gray. For this analysis, V/L/I, S/T, N/Q, D/E, K/R and W/F are considered equal. Residues shown are: for human IκBα aa 66-287 (SEQ ID NO: 12), for human Bcl-3 aa 31-278 (SEQ ID NO: 9), for murine IκBζ aa 292-629 (SEQ ID NO: 8), for human p105 aa 522-756 (SEQ ID NO: 11) and for murine p100 aa 467-705 (SEQ ID NO: 10).

Please replace the paragraph at page 43, lines 17 through 26 with the following amended paragraph:

FTOC were set up as described. On day 4, phosphorothioate oligonucleotides (sense, 5'CCCCTGGTGATGGAGGACTCT3' (SEQ ID NO: 6), or antisense, 5'AGAGTCCTCCATCACCAGGGG3' (SEQ ID NO: 7) from MWG Biotech, Inc.) were added at 200 μg/ml. After 12-19 h, VSV8 peptide was added to some FTOC at 300 μM. After 4 more hours, thymic lobes were harvested and analyzed by FACS. Thymocytes were stained at ~5 x 10^6

cells per ml in PBS-2% FCS-0.05% NaN3 containing the antibodies at saturating concentrations. The antibodies were anti-CD8α-FITC (53-6.7) and anti-CD4-PE (RM4.5) from Pharmingen. The phenotypes and proportions of thymocyte subsets were analyzed by two-color flow cytometry using a FACScan (Becton Dickinson) and the CellQuest program. Dead cells were excluded by gating.

COPY

REMARKS

The specification is amended to insert sequence identifiers (SEQ ID NOS), as appropriate. No new matter is added.

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

By Deirdre E. Sanders

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Dated: July 26, 2004

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Initials: DES/VJT/dcm Docket No.: 1062.2002-004 Date: July 26, 2004

This is to acknowledge receipt of the following documents each filed under Certificate of Mailing Procedure 37 C.F.R. 1.8:

X Petition to Accept Color Drawings/Photographs Under 37 C.F.R. §1.84 and Amendment w/copy

X DRAWINGS [8] Sheets Total
- X black/white photocopies
- X 3 sets color photos
- ___ Replacement Sheets
- ___ Annotated Sheets

___ Other_____

___ Pet Ext Time w/copy
X Check for $ 130.00
___ Authorization to charge fees

Applicants: Ellis L. Reinherz, *et al.*
Application No.: 10/783.994 Filed: February 20, 2004
Title: Identification of the IKBNS...
Date received by the PTO:

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Ellis L. Reinherz, Linda K. Clayton, Emma Fiorini, Pedro A. Reche and Ingo Schmitz		
Application No.:	10/783,994	Group:	1632
Filed:	February 20, 2004	Examiner:	Not yet assigned
Confirmation No.:	4354		
For:	IDENTIFICATION OF THE IKBNS PROTEIN AND ITS PRODUCTS		

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as First Class Mail in an envelope addressed to Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450

on 7-26-04 (Date) Dawn M Myers (Signature)

Dawn M Myers
Typed or printed name of person signing certificate

PETITION TO ACCEPT COLOR DRAWINGS/PHOTOGRAPHS UNDER 37 C.F.R. §1.84 AND AMENDMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Pursuant to 37 C.F.R. § 1.84, Applicants hereby petition for acceptance of color drawings of Figures 2 and 8, consisting of 2 sheets. Three (3) sets of color drawings of Figures 2 and 8 and a Transmittal of Replacement Drawings are being submitted concurrently herewith. In compliance with 37 C.F.R. § 1.84, a black and white photocopy of the enclosed color drawings is also enclosed.

COPY

REMARKS

Color drawings are the only practical medium by which to disclose the subject matter of Figures 2 and 8.

The petition fee as set forth in 37 C.F.R. § 1.17(h) in the amount of $130.00 is enclosed. Please charge any deficiency or credit any overpayment in the fees that may be due in this matter to Deposit Account No. 08-0380. A copy of this transmittal letter is enclosed for accounting purposes.

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

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Date: July 26, 2004

Molecular Cloning

A LABORATORY MANUAL

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Front cover: The electron micrograph of bacteriophage λ particles stained with uranyl acetate was digitized and assigned false color by computer. *Thomas R. Broker, Louise T. Chow, and James I. Garrels*

Back cover: E. coli DH1 with fimbriae was negatively stained with phosphotungstic acid and the electron micrograph was digitized and assigned false color by computer. *Jeffrey A. Engler, Thomas R. Broker, and James I. Garrels*

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HYBRIDIZATION OF DNA OR RNA IMMOBILIZED ON FILTERS TO RADIOACTIVE PROBES

There are many methods available to hybridize radioactive probes in solution to DNA or RNA immobilized on nitrocellulose filters. These methods differ in the following aspects:

the solvent and temperature used (68°C in aqueous solution or 42°C in 50% formamide);
the volume of solvent and the length of hybridization (large volumes for periods as long as 3 days or minimal volumes for times as short as 4 hours);
the degree and method of agitation (continuous shaking or stationary);
the concentration of the labeled probe and its specific activity;
the use of compounds, such as dextran sulfate, that increase the rate of reassociation of nucleic acids;
the stringency of washing following the hybridization.

Although the choice depends to a large extent on personal preference, we would like to offer the following guidelines.

1. Hybridization reactions in 50% formamide at 42°C are easier to set up, present less of an evaporation problem, and are less harsh on the filters than is hybridization at 68°C in an aqueous solution. The kinetics of the hybridization reaction in 80% formamide are approximately three to four times slower than in an aqueous solution (Casey and Davidson 1977). Assuming a linear relationship between the rate of hybridization and formamide concentration, the rate in 50% formamide should be two times slower than in an aqueous solution.

2. The smaller the volume of hybridization solvent, the better. The kinetics of nucleic acid reassociation are faster, and the amount of probe needed may be reduced so that the DNA on the filter acts as the driver for the reaction. All these are important parameters when detecting clones of low-abundance mRNAs. However, it is essential that sufficient liquid be present for the filters to remain at all times covered by a film of the hybridization solution.

3. Continual movement of the probe solution across the filter is unnecessary, even for a reaction driven by DNA immobilized on the filter. However, if a large number of filters are hybridized simultaneously, agitation is advisable in order to prevent the filters from adhering to each other.

4. The kinetics of the hybridization reaction are difficult to predict from theoretical considerations, partly because the exact concentration of the immobilized nucleic acid and its availability for hybridization are unknown.

When using probes made by nick translation of double-stranded DNA, the following rule of thumb is useful: Allow the hybridization to proceed for a time sufficient to enable the probe in solution to achieve 1–3× $C_o t_{1/2}$. In 10 ml of hybridization solution, 1 μg of a probe of 5-kb complexity will reach $C_o t_{1/2}$ in 2 hours. To determine the time of half-renaturation for any other probe, simply enter the appropriate values into the following equation:

$$\frac{1}{X} \times \frac{Y}{5} \times \frac{Z}{10} \times 2 = \text{number of hours to achieve } C_o t_{1/2}$$

where,

X = the weight of probe added (in μg)
Y = its complexity (for most probes, complexity is proportional to the length of the probe in kb)
Z = the volume of the reaction (in ml)

After hybridization for 3× $C_o t_{1/2}$ has been reached, the amount of the probe available for additional hybridization to the filter is negligible. For single-stranded cDNA probes, the hybridization time may be shortened since the lack of a competing DNA strand in solution favors hybridization to DNA bound to the filter.

5. In the presence of dextran sulfate, the rate of association of nucleic acids is accelerated because the nucleic acids are excluded from the volume of the solution occupied by the polymer. Their effective concentration is therefore increased. The rate of association reportedly increases 10-fold in the presence of 10% dextran sulfate (Wahl et al. 1979).

   Although dextran sulfate is useful in circumstances where the rate of hybridization is the limiting factor in detecting sequences of interest, it is unnecessary for most purposes. It is also difficult to handle because of its viscosity and sometimes can lead to high backgrounds.

6. In general, the washing conditions should be as stringent as possible; i.e., a combination of temperature and salt concentration should be chosen that is slightly (5°C) below the T_m of the hybrid under study. The temperature and salt conditions can often be determined empirically in preliminary experiments where Southern blots (see pages 382ff) of genomic DNA are hybridized to the probe of interest and then washed under conditions of different stringency.